PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference DUNBY/P30950PC	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/GB2004/003096	International filing date *(day/month/year)* 16.07.2004	Priority date *(day/month/year)* 17.07.2003
International Patent Classification (IPC) or national classification and IPC C07K14/82, C12N9/12		
Applicant UNIVERSITY OF DUNDEE et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 13 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 6 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26.07.2005	19.12.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Smalt, R Telephone No. +31 70 340-4275

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-162	as originally filed
Claims, Numbers	
1-35	filed with telefax on 18.11.2005
Drawings, Sheets	
1/31-31/31	as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 36-39
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/003096

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

   see separate sheet

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 25,26,30,33-35 and claims 31 and 32 partially

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 25,26,30,33-35 and claims 31 and 32 partially

   ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

   ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

   ☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☐ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☐ neither restricted nor paid additional fees.
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
   - ☐ complied with.
   - ☒ not complied with for the following reasons:

     see separate sheet
4. Consequently, this report has been established in respect of the following parts of the international application:
   - ☐ all parts.
   - ☒ the parts relating to claims Nos. 1-24,27-29 and 31 and 32 partially .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1,2,6-14,19,20,22-30
	No:	Claims	3-5,15-18,21,31,32
Inventive step (IS)	Yes:	Claims	6-14,25-28,30
	No:	Claims	1-5,15-24,29,31,32
Industrial applicability (IA)	Yes:	Claims	1-32
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

   and / or

2. Non-written disclosures (Rule 70.9)

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

The following documents (D) are cited in this communication; their numbering will be adhered to during the rest of the procedure:

D1: DATABASE WPI Section Ch, Week 200222 Derwent Publications Ltd., London, GB; Class B04, AN 2002-171818 XP002315199 & WO 02/06520 A1 (CHUGAI RES INST MOLECULAR MEDICINE INC) 24 January 2002 (2002-01-24)

D2: BOUDEAU J ET AL: "LKB1, a protein kinase regulating cell proliferation and polarity" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 546, no. 1, 3 July 2003 (2003-07-03), pages 159-165, XP004433636 ISSN: 0014-5793

D3: HAWLEY S A ET AL: "Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase." THE JOURNAL OF BIOLOGICAL CHEMISTRY. 1 NOV 1996, vol. 271, no. 44, 1 November 1996 (1996-11-01), pages 27879-27887, XP002315194 ISSN: 0021-9258

D4: BAAS A F ET AL: "Activation of the tumour suppressor kinase LKB1 by the STE20-like pseudokinase STRAD" EMBO JOURNAL, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 22, no. 12, 16 June 2003 (2003-06-16), pages 3062-3072, XP002298130 ISSN: 0261-4189

D5: BEAULOYE C ET AL: "Insulin antagonizes AMP-activated protein kinase activation by ischemia or anoxia in rat hearts, without affecting total adenine nucleotides" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 505, no. 3, 21 September 2001 (2001-09-21), pages 348-352, XP004309604 ISSN: 0014-5793

D6: HAWLEY SIMON A ET AL: "Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade." JOURNAL OF BIOLOGY (ONLINE) 2003, vol. 2, no. 4, 24 September 2003 (2003-09-24), page 28, XP002298131 ISSN: 1475-4924

D7: SHAW R J ET AL: "The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 101, no. 10, 9 March 2004 (2004-03-09), pages 3329-3335, XP002298134 ISSN: 0027-8424

D8: WO 2004/113562 A1 (MEDICAL RES COUNCIL [GB]; UNIV COLUMBIA [US];

CARLING DAVID [GB]; WOOD) 29 December 2004 (2004-12-29)

D9: BOUDEAU JÉRÔME ET AL: "MO25alpha/beta interact with STRADalpha/beta enhancing their ability to bind, activate and localize LKB1 in the cytoplasm." THE EMBO JOURNAL. 1 OCT 2003, vol. 22, no. 19, 1 October 2003 (2003-10-01), pages 5102-5114, XP002315196 ISSN: 0261-4189

D10: HONG SEUNG-PYO ET AL: "Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA. 22 JUL 2003, vol. 100, no. 15, 22 July 2003 (2003-07-22), pages 8839-8843, XP002315197 ISSN: 0027-8424

D11: SUTHERLAND CATHERINE M ET AL: "Elm1p is one of three upstream kinases for the Saccharomyces cerevisiae SNF1 complex." CURRENT BIOLOGY : CB. 5 AUG 2003, vol. 13, no. 15, 5 August 2003 (2003-08-05), pages 1299-1305, XP002315198 ISSN: 0960-9822

D12: WOODS A ET AL: "LKB1 is the upstream kinase in the AMP-activated protein kinase cascade" CURRENT BIOLOGY, CURRENT SCIENCE,, GB, vol. 13, no. 22, 11 November 2003 (2003-11-11), pages 2004-2008, XP002298132 ISSN: 0960-9822§§

D13: LIZCANO J M ET AL: "LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1" EMBO JOURNAL, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 23, no. 4, 25 February 2004 (2004-02-25), pages 833-843, XP002298133 ISSN: 0261-4189

D14: HARDIE D G: "THE AMP-ACTIVATED PROTEIN KINASE PATHWAY - NEW PLAYERS UPSTEAM AND DOWNSTREAM" JOURNAL OF CELL SCIENCE, CAMBRIDGE UNIVERSITY PRESS, LONDON, GB, vol. 117, no. PART 23, 2004, pages 5479-5487, XP008040901 ISSN: 0021-9533

Re: II

The position with regards to the invoked priorities can roughly be summarised as follows: the application as filed appears to be identical in every respect to the priority document of 20 December 2003. This priority differs from the earlier document of 17 July 2003 in that it has the following additions: page 40, line 14 to page 43, line 14; figure legends to figures 21-30 (pages 55-62); example 4 (pages 122-162), claims 32-39 (pages 166-168) and

figures 21-31.

This means that any subject-matter, which is not entitled to the first priority, will be assessed also in the light of D6 and D9-D12, since they were made public before the second priority date, and hence constitute full prior art.

Re: III & IV

Claims for which no search was performed cannot be the subject of examination according to Rule 66.1(e) PCT.

No searched was performed for claims 25,26, 33-35 completely, and claims 30 and 31 partially because the subject-matter of those claims does not form a unitary invention with the main invention (i.e. that mentioned first in the claim):

The present application discloses the formation of a complex of the kinase LKB1 with STRAD and MO25, and finds that the kinase activity for AMPK is enhanced by the cofactors when compared to LKB1 alone. D2 shows an interaction between LKB1 and STRAD, whereby STRAD is phosphorylated by LKB1, and the use of either of these sequences in the diagnosis of PJS. It is furthermore known from the prior art, as acknowledged in the application, and supported by references cited therein, that a number of drugs for treatment of diabetes act through AMPK. The fact that AMPK is phosphorylated and activated at Thr172 is commonly known at the priority date of the present application, see e.g. D6.

In the light of this prior art, a first problem underlying the present application has been defined as the provision of further complexes of LBK1 and uses thereof.
The solution lies in the provision of a complex comprising LKB1, STRAD, and MO25, and its use in the identification of modulators of the kinase activity of this complex towards AMPK subfamily members.

In the light of the prior art, a further problem underlying the invention has been defined as the provision of further methods for identifying binding partners of MO25, the solution being the method provided in claims 25 and 26.

In the light of the prior art, a third problem underlying the invention has been defined as the provision of further methods for the treatment of diabetes and/or obesity, the solution lying in the use of AMPK subfamily members.

In the light of the prior art, a fourth problem underlying the invention has been defined as the provision of further LKB1 substrates and antibodies thereto, the solution lying in the subject-matter essentially as described in present claims 33-35.

A number of sub-groups have been identified above under head-group 1, which do not necessarily form a unitary invention with the subject-matter of the first sub-group, but which could all be searched with little or no additional search effort with the first invention, in part because they are conceptually very closely linked thereto.

In view of the fact that complexes of LKB1 and STRAD are already known, and that the modulation of AMPK in the treatment of diabetes and obesity was also known, and that AMPK was known to be phosphorylated at position Thr172 during activation, and that LBK1 substrates were therefore also known, due to the essential difference between the four problems and their solutions, and since no other special technical feature, common to these solutions could be distinguished, the ISA is of the opinion that there is no single inventive concept underlying the plurality of claimed inventions of the present application within the sense of Rule 13.1 PCT. Consequently there is a lack of unity and the different inventions, not belonging to a common inventive concept, have been formulated in supplemental sheet B to the international search report as the different subjects on the communication pursuant to Art. 17(3)(a) PCT.

In view of the priority situation discussed above under item II, D6 has to be regarded as full prior art for claims 31-35, of which claims 31 and 32 in as far as they refer to claims 1, 20, 22 or 29 (i.e. not to the extent it refers to claim 30) are presently examined in view of the partial international search due to lack of unity. D6 describes both the method of claim 29 in as far as it relates to cells (see figure 7 and the section describing the experiment starting at the bottom of the right-hand column on page 28.8) and the kits of parts of claim 22. As these claims enjoy the first priority, D6 is not relevant. However, claims 31 and 32 do not enjoy the first priority, and D6 predates the second priority. D6 discloses both the use of AMPKα1 and AMPKα2 in both the cells and the kits of parts, and suggests the use

of eight of the AMPK-subfamily members listed in claim 32. Said aspects of claims 31 and 32 can hence not be considered to represent a common inventive concept or a common or corresponding special technical feature in the sense of Rule 13.1 and 13.2 PCT, respectively. Consequently there is a lack of unity between the methods featuring the individual AMPK-subfamily members as detailed in claims 31 and 32 in accordance with Art.34(3)(a) PCT. On the relevant second priority date none of these inventions is considered to have a unitary link with the first invention identified above as being the subject of the present substantive examination.

Re: V

Novelty

1. Amended claim 1 has been made new over D1 by incorporating the additional features of previous dependent claim 2.

2. D3 describes the purification from rat liver of an AMPKK complex, later characterized as a complex of LKB1, STRAD and MO25: see D15, page 5483, left-hand column, end of the second paragraph. In view of the applicant's argumentation, it can be accepted that over-expressed has an established and verifiable meaning in as far as it refers to a host cell. However, in an isolated protein or complex it is impossible to distinguish those isolated from nature from those derived from a host cell overexpressing the protein of one or more of the components of the complex. The applicant is also referred to the considerations below under point VIII on the term recombinant. In view thereof, present claims 3-5,15-18 (in as far as it relates to a preparation) and 21 are still considered to be anticipated by D3 in the sense of Art.33(2) PCT.

3. In view of the above detailed discussion of the disclosures in D6 (see last two paragraph under item III & IV regarding unity), the subject-matter of present claims 31 and 32 are not new over D6 in the sense of Art.33(2) PCT. Further to the applicant's comments, it is agreed that the method of present claim 29 is not disclosed by D6. An objection to claim 29 for lack of inventive step under Art.33(3) PCT in light of D6 has however been raised instead below. Furthermore, D6 is considered to disclose, at least

implicitly, a kit of parts as defined in present claim 22; the components of the kit have clearly been functionally linked prior to the filing date of the application. The novelty objections to claim 31 and 32 are therefore maintained in as far as these claims refer to the kit of parts defined in claim 22.

4. D12 is also published in time to be relevant prior art for claims 31 and 32. It describes the link between LKB1 (which corresponds to the activity of AMPKK isolated from rat liver) and AMPK. It also discloses cells in which the LKB1 activity is blocked and phosphorylation of AMPK is thereby abolished, which fall under the scope of claim 29, as well as compositions which fall under the definition of the kit of parts of claim 22 (although not specified in D12, AMPKK in fact corresponds to the complex of LKB1, STRAD and MO25; see e.g. D9, also available in time for claims 31 and 32). The subject-matter of claim 31 is not new over D12 in the sense of Art.33(2) PCT. In response to the applicant's submissions regarding this objection, it is considered that contrary to the disclosure in D6, the subject-matter described in D12 is so explicitly referring to the investigation of AMPK regulation that both the kit of claim 22 and the method of claim 29 are considered to be implicitly disclosed.

Inventive step

1. In view of the teaching in D3 regarding the purified AMPKK complex and its kinase activity for Thr172 of AMPK, the skilled person is not expected to experience any difficulties in devising a method for identifying modulators of this activity, and furthermore has a clear incentive to do so. In response to the first paragraph under the heading inventive step of the applicant's fax of 18.11.'05, the examiner would like to clarify that in the question whether the skilled person could or would two aspects have to be considered. Firstly it was established that he/she had an incentive. A <u>further</u> consideration is whether the skilled person would be able to put the envisaged method to practice. Only if both considerations can be answered in the positive, which in the case at hand they are, may one conclude that the subject-matter is not inventive over the prior art teaching. The reader of D3 does not know the composition of the AMPKK complex, but since it is available in a purified form, that would not appear to hamper development of the method in any way. Since the complex is in fact one as described in claims 1,2,17, and 22 these too are not considered inventive in the sense of Art.33(3) PCT, nor is the method of claim 19 and 20

to the extent that it relates to claim 19. In response to the comments to this objection by the applicant in the letter of 21.7.'05 it is pointed out that the claims objected to do not use LKB1 in purified form, but rather specify complexes which cover those occurring in nature, as purified in D3. The skilled person would have used these complexes, and whether he/she knew that the composition was in fact identical to that defined in the present claims makes no difference; that is what he/she would have used, which is identical to what is claimed, and that is hence not inventive. Similarly, since the effect of AICA riboside, metformin and phenformin on AMPK activation was commonly known in the art, it is not considered inventive to use the complex described and purified in D3 for comparative testing of this response, as described in present claim 29. It is common knowledge that AMPK is activated by phosphorylation at Thr172, as is repeated in D3, see also e.g. D5. The members of the AMPK family are also commonly known, as is their functional equivalence to AMPKα1 and the corresponding position at which they are phosphorylated in activation. The use of this position and/or of the family members is not considered to involve an inventive step in light of D3; claims 31 and 32 are not considered to meet the requirements of Art.33(3) PCT. In response to the applicants comments to this objection in the latter of 21.7.'05, the question one should pose is what the skilled person would do when confronted with the problem solved by the claimed subject-matter when compared to the prior art. The prior art provides the knowledge that AMPK is phosphorylated at Thr172 and activated thereby. The difference between the claimed mutants lacking Thr at position 172 or that corresponding thereto in the other AMPK-subfamily members and the prior art is that the mutants are no longer activatable. The problem solved is hence the provision of non-activatable mutants of the proteins previously known. The solution lies in the mutation of the critical Thr residue. Since the identity and location of the critical residues was known for all claimed mutant proteins, it is considered that the solution is one that the skilled person WOULD have come up with, and is therefore not considered as inventive.

2. Further to paragraph 5 under the section novelty above, claim 32 is not considered inventive over D12, as the claimed alternative subfamily members of AMPKα are all known to be functional equivalents at the relevant time from e.g. D6.

3. Further to § 3 under the heading novelty above, it is indeed agreed that D6 does not disclose screening methods for modulators other than AICA riboside, metformin and phenformin, and hence does not affect the novelty of claim 29. However, from the fact that

the above modulators are known as such, is can be concluded that screening method for their identification are known. What D6 discloses is a method to demonstrate the effect of the previously known modulators. It would be immediately obvious to the skilled person that these same methods can be used to screen for and identify as yet unknown modulators with comparable properties. The subject-matter of claim 29 can therefore not be considered to involve an inventive step over D6 in the sense of Art.33(3) EPC.

Re: VI

Some national and/or regional laws have provisions for novelty objections on the basis of patent documents published after the priority date(s) of the application under examination, but with a priority before that/those date(s). For the present application, D8 is such a document, which could become relevant for the purpose of novelty in the national/regional phase for the claims as indicated in the international search report.

Re: VIII

1. The term recombinant can be used to describe a coding DNA, because this can be recognized as being different from the non-recombinant homologue, e.g. because it lies on a plasmid, it has a heterologous promoter, etc. However, the protein expressed from such recombinant coding sequence is identical to and indistinguishable from the protein expressed from the natural coding sequence. The applicants arguments on this point relate to the recombinant gene or coding sequence, whereas the objection raised is clearly limited to the protein and complexes thereof, which are indistinguishable and hence identical to those known from the art, and they hence lack novelty.

2. Present claims 23 and 24 relate to a method for expressing LKB1, where LKB1 was known in the art. Such a method can thus not be considered as inventive. However, what appears to be the intended scope of the claim is a method to produce AMPKK complex by overexpressing LKB1 in a cell which also expresses STRAD and MO25. Such a claim would be allowable.

CLAIMS

1. A method for identifying a compound for use in modulating, for example promoting, the activation or phosphorylation of AMPK (AMP-activated protein kinase) or AMPK subfamily member in a cell, the method comprising the steps of (1) determining whether a test compound modulates, for example promotes, the protein kinase activity of LKB1 and (2) selecting a compound which modulates, for example promotes, the protein kinase activity of LKB1, wherein the LKB1 is in a preparation with STRAD and/or MO25.

2. The method of claim 1 wherein the LKB1, STRAD or MO25 is recombinant and which is expressed from a recombinant nucleic acid.

3. A purified preparation comprising LKB1, STRAD and recombinant MO25 expressed from a recombinant nucleic acid.

4. The preparation of claim 4 comprising recombinant LKB1 expressed from a recombinant nucleic acid.

5. The preparation of claim 3 or 4 comprising recombinant STRAD expressed from a recombinant nucleic acid.

6. A cell capable of expressing LKB1, STRAD and overexpressed or recombinant MO25 expressed from a recombinant nucleic acid.

7. The cell of claim 6 comprising a recombinant nucleic acid encoding MO25.

8. The cell of claim 6 or 7 comprising a recombinant nucleic acid encoding LKB1.

9. The cell of any one of claims 6 to 8 comprising a recombinant nucleic acid encoding STRAD.

10. A cell comprising LKB1, STRAD and overexpressed or recombinant MO25 expressed from a recombinant nucleic acid.

11. A cell according to claim 10 comprising recombinant LKB1 expressed from a recombinant nucleic acid.

12. A cell according to claim 10 or 11 comprising recombinant STRAD expressed from a recombinant nucleic acid.

13. A cell according to any one of claims 10 to 12 wherein the cell is a cell according to any one of claims 6 to 9.

14. A method for making a preparation according to any one of claims 3 to 5 comprising the step of purifying the preparation from a cell according to any one of claims 10 to 13.

15. A preparation obtainable by the method of claim 14.

16. The preparation of any one of claims 3 to 5 or 15 wherein the LKB1:STRAD:MO25 ratios are 1:1:1.

17. The method of claim 1 or 2 wherein the LKB1 is in a preparation as defined in any one of claims 3 to 5 or 16 or a preparation obtained by or obtainable by the method of claim 14 or in a cell as defined in any one of claims 6 to 13.

18. The preparation or method of any one of claims 1 to 5, 14 to 17 wherein the preparation comprises a complex comprising the LKB1, STRAD and MO25.

19. A method for identifying a compound for modulating cellular LKB1 activity, the method comprising the steps of (1) determining whether a test compound modulates the LKB1 protein kinase activity of a preparation or complex as defined in any one of claims 3 to 5, 15, 16 or 18 or in a cell as defined in any one of claims 6 to 13 and (2) selecting a compound which modulates the said LKB1 protein kinase activity.

20. The method of claim 1 or claim 19 wherein the LKB1 protein kinase activity is measured using AMPK or AMPK subfamily member or a fragment either thereof as the substrate.

21. A kit of parts comprising LKB1 or a recombinant polynucleotide encoding LKB1, STRAD or a recombinant polynucleotide encoding STRAD, and MO25 or a recombinant polynucleotide encoding MO25.

22. A kit of parts comprising (1) AMPK or AMPK subfamily member, or recombinant polynucleotide encoding AMPK or AMPK subfamily member or a fragment thereof and (2) a kit of parts as defined in claim 21 or a preparation or complex as defined in any one of claims 3 to 5, 15, 16 or 18 or a cell as defined in any one of claims 6 to 13.

23. A method for overexpressing LKB1 comprising the steps of (1) selecting a cell type in which to overexpress LKB1, comprising the step of determining whether the cell type is one that expresses STRAD and/or MO25 (2) overexpressing LKB1 in the selected cell type.

24. A method for preparing LKB1 comprising the steps of (1) overexpressing LKB1 in a cell using a method according to claim 23 and (2) preparing LKB1 from the cell.

25. A method for identifying a putative binding partner for MO25 comprising the steps of (1) providing an amino acid sequence of at least the C-terminal three amino acids of a test putative binding partner (2) selecting a putative binding partner having the C-terminal amino acid sequence Trp-Glu/Asp-Phe.

26. The method of claim 25 further comprising the step of determining that the selected putative binding partner binds to MO25.

27. A method for identifying a genetic difference associated with PJS (Peutz-Jeghers Syndrome) comprising the steps of (1) investigating the sequence of a gene encoding a MO25 isoform in at least one patient having PJS (2) identifying any difference between the said patient sequence and equivalent sequence from an individual without PJS.

28. A method for determining whether an individual is susceptible to PJS comprising the steps of determining whether the test individual has a genetic difference identified as associated with PJS by a method according to claim 27.

29. A method for identifying a compound which activates AMPK or AMPK subfamily member by a similar mechanism to metformin or phenformin or AICA riboside in which the effect of a test compound on the activation of AMPK or AMPK subfamily member by a preparation or complex as defined in any one of claims 3 to 5, 15, 16 or 18 or a cell as defined in any one of claims 6 to 13 is compared with the effect of metformin or phenformin or AICA riboside on the activation of AMPK or AMPK subfamily member and a compound with a similar effect is selected.

30. Use of an AMPK subfamily member or polynucleotide encoding an AMPK subfamily member in the manufacture of a medicament for treating diabetes or obesity.

31. The method of any one of claims 1, 20 or 29, kit of parts of claim 22, or use of claim 30 wherein the AMPK subfamily member is or comprises an AMPKα1 or AMPKα2 polypeptide.

32. The method of any one of claims 1, 20, 29, kit of parts of claim 22 or use of claim 30 wherein the AMPK subfamily member is or comprises a NUAK1, NUAK2, BRSK1, BRSK2, SIK, QIK, QSK, MARK1, MARK2, MARK3, MARK4 or MELK polypeptide.

33. A peptide substrate for LKB1 comprising the amino acid sequence LSNLYHQGKFLQTFCGSPLY or FGNFYKSGEPLSTWCGSPPY or LSNMMSDGEFLRTSCGSPNY or MASLQVGDSLLETSCGSPHY or FSNEFTVGGKLDTFCGSPPY or AKPKGNKDYHLQTCCGSLAY; or a said sequence with from one to four substitutions therein at any position other than the underlined residue and/or a conservative substitution at the underlined residue; or at least ten contiguous residues of a said sequence encompassing the underlined residue.

34. A peptide substrate for LKB1 consisting of the amino acid sequence LSNLYHQGKFLQTFCGSPLY or LSNLYHQGKFLQTFCGSPLYRRR or SNLYHQGKFLQTFCGSPLY or SNLYHQGKFLQTFCGSPLYRRR or LSNLYHQGKFLQTFCGSPLY or LSNLYHQGKFLQTFCGSPLYRRR or FGNFYKSGEPLSTWCGSPPY or FGNFYKSGEPLSTWCGSPPYRRR or LSNMMSDGEFLRTSCGSPNY or LSNMMSDGEFLRTSCGSPNYRRR or MASLQVGDSLLETSCGSPHY or MASLQVGDSLLETSCGSPHYRRR or

FSNEFTVGGKLDTFCGSPPY or FSNEFTVGGKLDTFCGSPPYRRR or AKPKGNKDYHLQTCCGSLAY or AKPKGNKDYHLQTCCGSLAYRRR.

35. An antibody reactive with a peptide antigen having the amino acid sequence MVAGLTLGKGPESPDGDVS (residues 1-20 of human BRSK1), LSWGAGLKGQKVATSYESSL (residues 655-674 of human BRSK2), MEGAAAPVAGDRPDLGLGAPG (residues 1-21 of human NUAK1), TDCQEVTATYRQALRVCSKLT (residues 653-673 of human NUAK2), MVMADGPRHLQRGPVRVGFYD (residues 1-21 of human QIK), MVIMSEFSADPAGQGQGQQK (residues 1-20 of human SIK), GDCEMEDLMPCSLGTFVLVQ (residues 765-784 of human SIK), TDILLSYKHPEVSFSMEQAGV (residues 1349-1369 of human QSK), SGTSIAFKNIASKIANELKL (residues 776-795 of human MARK1), MSSRTVLAPGNDRNSDTHGT (residues 1-20 of human MARK4), MKDYDELLKYYELHETIGTG (residues 1-20 of human MELK), CTSPPDSFLDDHHLTR (residues 344-358 of rat AMPKα1), CDPMKRATIKDIRE (residues 252 to 264 of rat AMPKα1).